ACUTE CORONARY SYNDROMES

Association of haematological indices with the degree of microvascular injury in patients with acute anterior wall myocardial infarction treated with primary percutaneous coronary intervention

Murat Sezer, Irem Okcular, Taner Goren, Huseyin Oflaz, Yılmaz Nisanci, Berrin Umman, Fehmi Mercanoglu, Ahmet K Bilge, Mehmet Meric, Sabahattin Umman

Heart 2007;**93**:313–318. doi: 10.1136/hrt.2006.094763

See end of article for authors' affiliations

Correspondence to: Dr M Sezer, Department of Cardiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; sezerm@istanbul.edu.tr; msezer@superonline.com

Received 4 April 2006
Accepted 21 July 2006
Published Online First 29 August 2006

Background: In acute myocardial infarction (AMI), increased neutrophil count has been associated with more severe coronary artery disease and larger infarct size. Increased mean platelet volume (MPV) is also associated with poor clinical outcome and impaired angiographic reperfusion in patients with AMI. However, the associations of neutrophil count and MPV with the indices of tissue level reperfusion were not fully elucidated.

Aim: To elucidate the relationship between baseline neutrophil count and MPV on presentation and microvascular injury in patients with anterior AMI treated with primary percutaneous coronary intervention (pPCI).

Methods: 41 patients with anterior wall AMI treated successfully with pPCI were included. The leucocyte count, neutrophil count and MPV were obtained on admission, and the percentage of neutrophils was calculated. After PCI thrombolysis in myocardial infarction, grade 3 flow was established in all patients. The coronary flow velocity pattern (diastolic deceleration time (DDT)) was examined with transthoracic echocardiography and measured intracoronary pressures with fibreoptic pressure–temperature sensor-tipped guidewire in the left anterior descending artery within 48 h after pPCI. Thermodilution-derived coronary flow reserve (CFR) was calculated. Index of microvascular resistance (IMR) was defined as simultaneously measured distal coronary pressure divided by the inverse of the thermodilution-derived hyperaemic mean transit time. Subsequently, a short compliant balloon was placed in the stented segment and inflated to measure coronary wedge pressure (CWP).

Results: Higher neutrophil counts were strongly associated with higher IMR ($r=0.86$, $p<0.001$), lower CFR ($r=-0.60$, $p<0.001$), shorter DDT ($r=-0.73$, $p<0.001$) and higher CWP ($r=0.73$, $p<0.001$). Likewise, there were significant correlations among the percentage of neutrophils and CFR ($r=-0.34$, $p=0.02$), IMR ($r=0.46$, $p=0.002$), DDT ($r=-0.36$, $p=0.01$) and CWP ($r=0.49$, $p=0.001$). Relationships among leucocyte count and IMR ($r=0.38$, $p=0.01$), CFR ($r=-0.33$, $p=0.03$), DDT ($r=-0.36$, $p=0.01$) and CWP ($r=0.32$, $p=0.026$) were slightly significant. Higher neutrophil count remained independently associated with indices of microvascular perfusion in multivariable models controlling for age, smoking habits and time to treatment. Also, higher MPV on admission was strongly associated with higher IMR ($r=0.89$, $p<0.001$), steeper DDT ($r=-0.64$, $p<0.001$), lower CFR ($r=-0.43$, $p=0.004$) and higher CWP ($r=0.77$, $p<0.001$).

Conclusion: Absolute and relative neutrophilia and higher MPV on admission were independently associated with impaired microvascular perfusion in patients with anterior AMI treated with pPCI. It is possible that neutrophilia and high MPV are simple surrogate markers of worse microvascular injury in patients with AMI.

The leucocyte and neutrophil counts on admission are strong and independent predictors of increased morbidity and mortality in patients with acute myocardial infarction (AMI).[1] The major component of leucocytosis associated with acute coronary syndromes is the increase in neutrophil counts. The potential roles of neutrophils in promoting coagulation, increasing microvascular permeability and mediating ischaemia-reperfusion injury in acute coronary syndromes have been described previously.[2] Leucocyte intravascular plugging seems to play an important role in the pathophysiology of no-reflow phenomenon as well. Increase in the leucocyte and neutrophil counts in AMI have been associated with more severe coronary artery disease, larger infarct size and higher risk of acute ischaemic events.[3] However, the association of leucocyte and/or neutrophil counts with the indices of myocardial perfusion is less clear.

Another blood component—platelets—play a crucial role in the pathogenesis of acute coronary syndromes and also in the no-reflow phenomenon. Large platelets are denser,[4] aggregate more rapidly and express more glycoprotein Ib and glycoprotein IIb–IIIa receptors.[5] It has been shown that platelet size, measured as mean platelet volume (MPV), is associated with their activity. Large platelets are metabolically and enzymatically more active than small platelets and have higher thrombotic potential.[6] Increased MPV is also associated with

Abbreviations: AMI, acute myocardial infarction; CFR, coronary flow reserve; CWP, coronary wedge pressure; DDT, diastolic deceleration time; IMR, index of microvascular resistance; LAD, left anterior descending artery; MPV, mean platelet volume; pPCI, primary percutaneous coronary intervention

poor clinical outcome and impaired angiographic reperfusion in patients with AMI.[7 8]

Furthermore, degranulated platelets tend to aggregate with neutrophils and monocytes in vitro, and increased numbers of neutrophil–platelet and monocyte–platelet aggregates have been found in circulation in patients with coronary artery disease.[9 10] Also, platelets and neutrophils act synergistically in provoking post-reperfusion cardiac dysfunction (reperfusion injury).[11]

The goal of this study was to elucidate the relationship between baseline neutrophil (absolute and relative) counts and MPV on admission and the degree of microvascular injury in patients with anterior wall AMI successfully treated with primary percutaneous coronary intervention (pPCI).

METHODS

Patients

A total of 41 patients with anterior wall AMI treated successfully with pPCI were prospectively included. Inclusion criteria were (1) continuous chest pain that lasted >30 min within the preceding 12 h; (2) ST-segment increase of at least 1 mm in two contiguous leads on the 12-lead ECG; and (3) angiographically detected culprit coronary artery lesion deemed suitable for pPCI. After pPCI, all patients had thrombolysis in myocardial infaction grade 3 flow with no residual stenosis in infarct-related artery (left anterior descending artery (LAD)). The major exclusion criteria were the presence of additional pronounced lesions in the LAD distal to the culprit lesion and inability to identify diastolic flow echocardiographically in LAD.

Peri-interventional treatment

All patients received aspirin 300 mg and a loading dose of clopidogrel 600 mg at the beginning of the pPCI, and intracoronary unfractionated heparin 100 U/kg and tirofiban in a bolus of 0.1 μg/kg body weights in 3 min followed by a continuous infusion of 0.15 μg/kg/min for 12 h during the procedure. Written informed consent was obtained from all patients and study protocol was approved by our local institutional review board. The study was carried out according to the Declaration of Helsinki.

Laboratory analysis

In all cases, venous peripheral blood samples for the neutrophil count (absolute and relative) and MPV measurement were drawn before pPCI and before any treatment was given. Blood samples were taken into tubes containing dipotassium EDTA and measurements were taken on Beckmann Coulter HMX Hematology Analyzer. The assessment of MPV was made before clopidogrel, heparin or tirofiban administration. The percentage of neutrophils was defined as the neutrophil count divided by the leucocyte count.

Intracoronary haemodynamic measurements and angiographic analysis

Evaluation of microvascular condition was scheduled on day 2. Patients were recatheterised 40 (11) h after pPCI. After control coronary angiography, a 0.014 inch fiberoptic pressure–temperature sensor-tipped guidewire (Pressure wire sensor 5, Radi Medical Systems, Uppsala, Sweden) was advanced through the guiding catheter and positioned as distal as possible to the stented segment of the LAD. Proximal aortic and distal intracoronary pressures were recorded simultaneously. Papaverine was the hyperaemic agent used and was given at doses of 20 mg intracoronary bolus for left system. The mean transit time at rest and during hyperaemia were recorded after rapid injection of 3 ml of room-temperature saline through the guiding catheter as described previously. The hyperaemic and resting mean transit times were measured three times and averaged.[12] Thermodilution-derived coronary flow reserve (CFR) was calculated as the resting mean transit time divided by the hyperaemic mean transit time.[13] Index of microvascular resistance (IMR) was defined as simultaneously measured distal coronary pressure divided by the inverse of the thermodilution-derived hyperaemic mean transit time or, more simply, distal coronary pressure multiplied by the hyperaemic mean transit time (mm Hg seconds, or units (U); fig 1). Subsequently, a short compliant balloon (shorter than the stent length) was placed in the stented segment and inflated to measure CWP (mm Hg). Mean (CWPm) and phasic values of CWP were recorded simultaneously.

Analysis of coronary flow velocity pattern

Measurement of coronary flow in the LAD was performed echocardiographically just before intracoronary haemodynamic measurements with Vivid 7 Digital Ultrasound System (General Electrics) with a frequency of 7 MHz transducer. Coronary blood flow in the distal portion of the LAD was searched under the guidance of colour Doppler flow mapping. We placed a sample volume on the colour signal in the distal portion of coronary artery to record coronary flow velocity using the pulsed Doppler. From the coronary flow velocity spectrum, diastolic deceleration time (DDT, ms) was measured from the peak diastolic velocity to the point where the extrapolated line intersected the baseline as described in previous reports and was calculated as the mean of three continuous cardiac cycles.

Statistical analysis

All analyses were performed using SPSS V.11.0 for windows. All continuous variables were reported as mean (SD). Leucocyte and neutrophil counts were reported as values $\times 10^9$/l. Linear regression analysis was used to assess correlations between the variables. Two-sided t tests were used in the comparison of normally distributed continuous variables. The non-parametric Mann–Whitney U test was used when the data were not normally distributed. A p value <0.05 was considered significant. Multivariate regression models were built for microvascular perfusion indices and neutrophil count, percentage of neutrophils and MPV, adjusting for age, smoking habits and time to treatment.

RESULTS

Baseline characteristics

Table 1 lists the baseline clinical and angiographic characteristics of the patients. Table 2 shows the relationship between cell counts and various baseline clinical characteristics. Smokers had a significantly higher neutrophil count and higher percentage neutrophil count than non-smokers. Patients with time from symptom onset to pPCI more than the median (3.2 h) had both a higher neutrophil count and a higher percentage of neutrophils than patients with earlier time to treatment. Older patients had a slightly lower neutrophil count than younger patients. There were no significant differences in cell counts according to other baseline clinical characteristics.

MPV correlated with absolute neutrophil count ($r = 0.79$, $p<0.001$) and leucocyte count ($r = 0.40$, $p = 0.007$). Also, absolute neutrophil count ($r = 0.32$, $p = 0.01$) and leucocyte count ($r = 0.35$, $p = 0.009$) modestly correlated with baseline platelet counts.

Association of leucocytes with the degree of microvascular injury

The mean (SD) leucocyte count was 12.3 (3.2×10^3) μl. The mean neutrophil count was 8.9 (2.6×10^3) μl with a percentage of 76% (14%). Higher neutrophil count was strongly associated

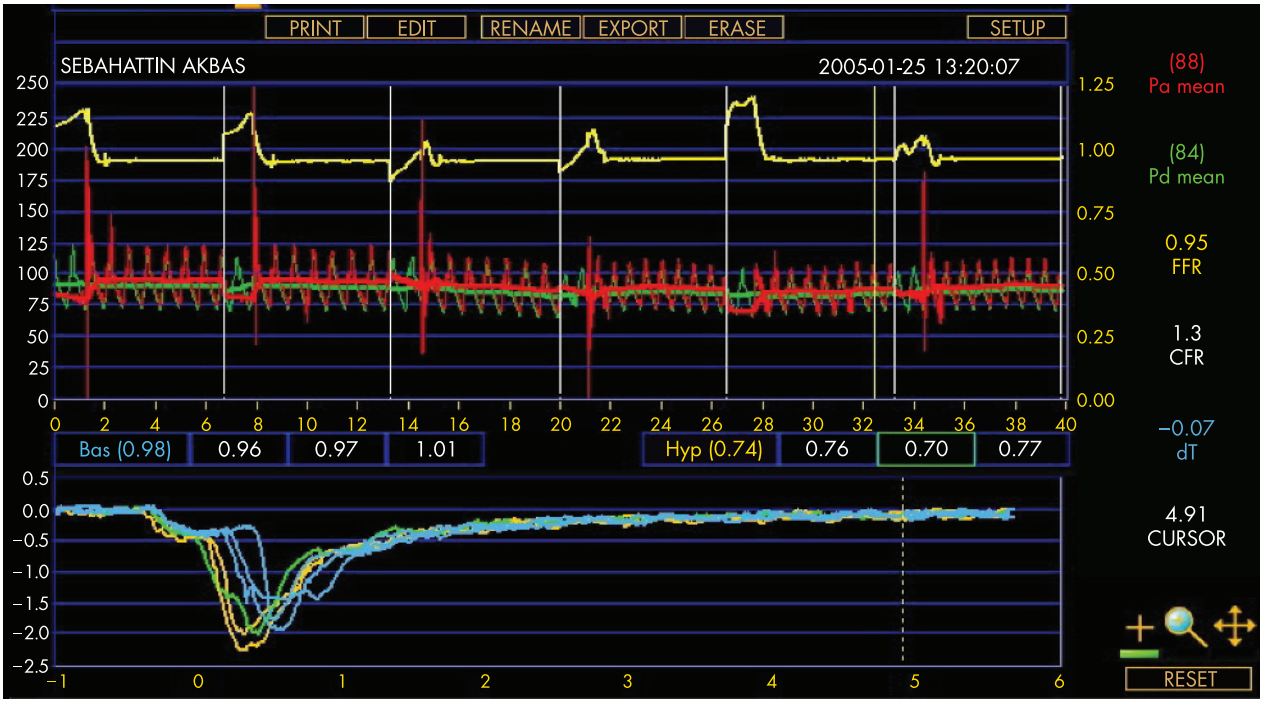

Figure 1 Intracoronary haemodynamic measurement.

with higher IMR (r = 0.86, p<0.001), lower CFR (r = −0.60, p<0.001), shorter DDT (r = −0.73, p<0.001) and higher CWP (r = 0.73, p<0.001; fig 2). Likewise, there were significant correlations among the percentage of neutrophils and CFR (r = −0.34, p = 0.02), IMR (r = 0.46, p = 0.002), DDT (r = −0.36, p = 0.01) and CWP (r = 0.49, p = 0.001). Relationships among leucocyte count and IMR (r = 0.38, p = 0.01), CFR (r = −0.33, p = 0.03), DDT (r = −0.36, p = 0.01) and CWP (r = 0.32, p = 0.026) were slightly significant.

Association of MPV with the degree of microvascular injury

The mean platelet count was 286×10^3 with the mean (SD) MPV 9.95 (0.98) fl. Higher MPV on admission was strongly associated with higher IMR (r = 0.89, p<0.001), steeper DDT (r = −0.64, p<0.001), lower CFR (r = −0.43, p = 0.004) and higher CWP (r = 0.77, p<0.001; fig 3).

Table 1 Baseline clinical and angiographic characteristics

Patients (n)	41
Mean (SD) age (years)	54 (6)
Smoking, n (%)	30 (73)
Diabetes mellitus, n (%)	13 (31)
Hypertension, n (%)	15 (36)
Dyslipidaemia, n (%)	30 (73)
History of preinfarction angina, n (%)	12 (29)
Infarct related artery, LAD, n (%)	41 (100)
Baseline TIMI flow 0/1, n (%)	41 (100)
Number of diseased vessels, n (%)	
1	30 (73)
2	6 (15)
3	3 (12)
Mean (SD) time from symptom onset to first balloon inflation (min)	260 (112)

Multivariate models

In multivariate models incorporating time to treatment, age and smoking habits, a higher neutrophil count remained independently associated with higher IMR (p = 0.006), higher CWP (p = 0.002), steeper DDT (p = 0.02) and lower CFR (p = 0.01). Likewise, a higher MPV remained independently associated with impaired microvascular perfusion as evidenced by higher IMR (p = 0.008), higher CWP (p = 0.01), steeper DDT (p = 0.01) and lower CFR (p = 0.02).

DISCUSSION

This study confirms and expands previous observations that an increased neutrophil count and MPV were associated with adverse microvascular outcomes in AMI. In our study, microvascular perfusion has been evaluated with quantitative and objective indices in patients with patent infarct-related artery, and the relationship between haematological parameters and microvascular perfusion parameters were shown. Our findings showed that neutrophil count (absolute and relative) and MPV on admission were strongly and independently associated with the degree of microvascular damage. An increased neutrophil count and a higher MPV were associated with impaired microvascular perfusion after pPCI, as evidenced consistently by higher IMR, lower CFR, steeper DDT slope and higher CWP.

The ideal time to measure microvascular function to determine the extent of myocardial necrosis is 48 h after reperfusion. At that time, dynamic changes in resting tissue perfusion have subsided, and the extent of no reflow correlates well with infarct size and denotes a region of irreversible tissue damage.[14 15] Therefore, we performed all analysis for interrogation of the microcirculation 48 h after pPCI with the assumption of that ideal timing. Differing from the previous studies in this field,[16] this study uses quantitative and sensitive indices to evaluate the integrity of the distal vascular bed. It has been shown that CWP is a sensitive parameter for estimating microvascular dysfunction in AMI.[17 18] CFR and DDT were also

Table 2 Relationships between clinical characteristics and haematological indices

		NC		% NC		MPV	
	n	$\times 10^9$/l	p Value	%	p Value	$\times 10^9$/l	p Value
Time to treatment	(+)23	8.7 (3.2)	0.01*	76.211	0.02*	10.1 (0.9)	NS
> Median	(−)18	7.3 (3.6)	67.1 (9)	9.4 (0.8)			
Men	(+)35	7.7 (2.7)	NS	74.4 (10.2)	NS	9.3 (0.7)	NS
	(−) 6	7.5 (3)	71.2 (11.2)	10.8 (0.8)			
Prior angina	(+)12	7.7 (3.3)	NS	69.3 (10.4)	NS	11.2 (0.9)	NS
	(−) 29	7.9 (2.9)	71.9 (9.9)	9.8 (0.8)			
Smoker	(+)30	8.5 (3.2)	0.03*	74.9 (10.2)	0.03*	9.5 (0.8)	NS
	(−)11	7.6 (3.3)	67.2 (12.1)	10.2 (0.8)			
Diabetes	(+)13	7.9 (2.9)	NS	72.2 (11.2)	NS	11.2 (1)	NS
	(−)28	7.7 (3)		71.3 (9.9)		9.5 (0.8)	
Dyslipidaemia	(+)30	8.2 (3.4)	NS	70.2 (11.9)	NS	10.9 (0.8)	NS
	(−)11	7.8 (2.6)		72.3 (12.2)		9.7 (0.7)	

MPV, mean platelet volume; NC, neutrophil count; NS, non-significant.

proved to be better markers for detection of microvascular integrity than other angiographic, electrocardiographic and enzymatic modalities.[19–21] We also measured IMR, which can be used as a means for directly interrogating and quantifying microcirculatory function.[22] All these sensitive, objective and quantitative parameters of microvascular perfusion consistently showed that higher neutrophil count and MPV on admission were associated with worse microvascular injury after successful pPCI.

Neutrophils and microvascular damage

In acute coronary syndromes, neutrophils are localised in ruptured plaques and there is evidence of activation of neutrophils throughout the coronary tree.[23] In addition to being implicated in plaque instability and rupture in acute coronary syndromes, platelet–neutrophil interactions may play a role in thrombus formation and perpetuation of coagulation. Neutrophils may also be linked to microvascular injury and myocardial dysfunction by mediating ischaemia and reperfusion injury. During reperfusion of ischaemic myocardium, neutrophils and platelets can plug capillaries in the coronary microcirculation, resulting in the no-reflow phenomenon, ventricular arrhythmia, and loss of coronary vascular reserve, infarct extension and even organ dysfunction.[24 25] After successful epicardial recanalisation, neutrophils can worsen

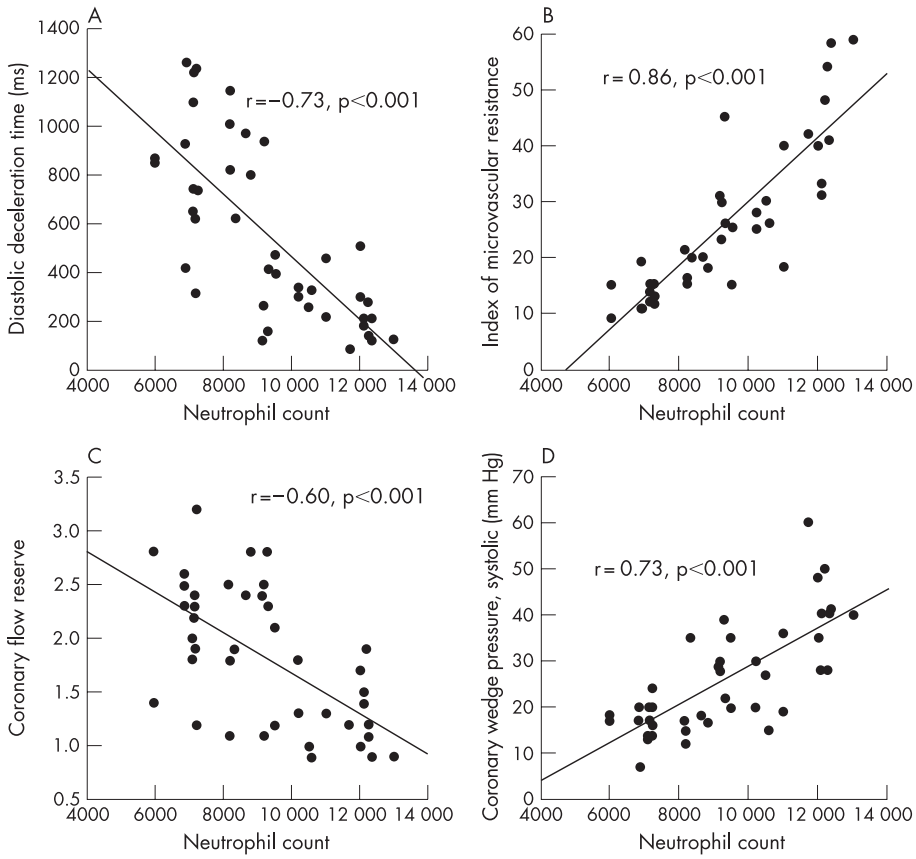

Figure 2 Relationships between absolute neutrophil count and indices of microvascular perfusion. (A) Diastolic deceleration time and neutrophil count. (D) Coronary wedge pressure, systolic and neutrophil count.

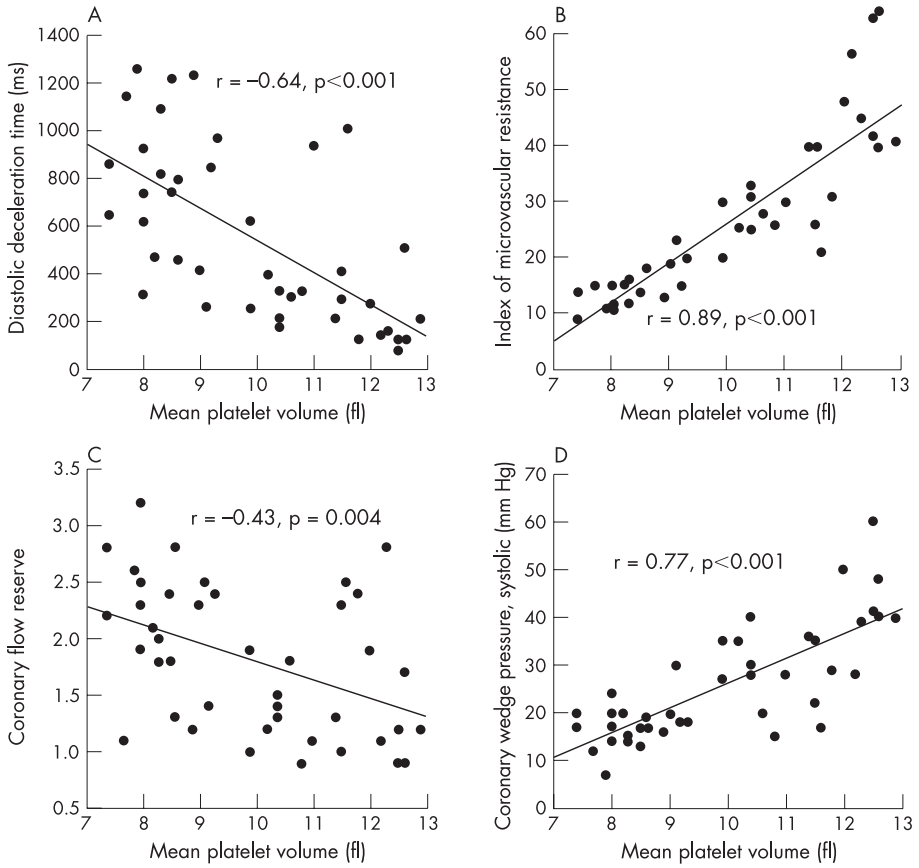

Figure 3 Relationships between mean platelet volume (MPV) and indices of microvascular perfusion. (A) Diastolic deceleration time and MPV. (D) Coronary wedge pressure and MPV.

microvascular reperfusion by adhering to the endothelium with platelets[26] and by releasing cytokines or other factors that reduce microvascular blood flow.[27] In animal studies of a neutrophil depletion model using anti-neutrophil agents, microvascular reperfusion injury was reduced, leading to smaller infarct sizes and better ventricular function.[28] In this study, we showed a close association between baseline neutrophilia and impaired microvascular perfusion as evidenced consistently by lower CFR, higher IMR, higher CWP and steeper DDT slope. This association could be the consequence of neutrophil-mediated damage on the endothelium of the coronary artery and arterioles and microvascular plugging as observed in animal models of ischaemia reperfusion.[29] Also, neutrophil-derived mediators such as oxygen free radicals and proteolytic enzymes can cause progressive decrease in coronary vascular endothelial function.[25] Engler *et al*[30] reported that more than half of the capillaries observed in an ischaemic reperfused myocardium were not perfused. These non-perfused microvessels in the no-reflow zone contained numerous adherent neutrophils.[31] All these possible mechanisms together may account for neutrophil-mediated damage of the coronary endothelium especially at the pre-arteriolar and/or arteriolar level (regulatory level of coronary resistance) and can cause changes in CFR and IMR.

Although we observed an association between time to treatment and neutrophilia as described previously,[32 33] the relationship between neutrophilia and impaired microvascular perfusion remained significant in multivariable analyses, incorporating time to treatment and age.

Platelet volume and microvascular damage

Circulating platelets are heterogeneous in size, density and reactivity.[34] Changes in these variables may be causal in acute coronary syndromes.[35] There is strong evidence indicating that MPV is an important biological variable[36] and that large platelets have a higher thrombotic potential. It has been shown that large platelets are metabolically and enzymatically more active than small platelets.[6] Post-AMI microvascular impairment in the presence of infarct-related artery patency may be attributable to a number of factors, including distal embolisation of small platelet aggregates,[37] release of vasoconstrictive mediators and direct platelet–leucocyte interaction with the blood vessel wall.[38] Recently, Neumann *et al*[10] showed that leucocyte–platelet binding is increased in patients with AMI. Also, it has been known that platelets and neutrophils act synergistically in provoking post-reperfusion cardiac dysfunction (reperfusion injury).[11] Higher MPV may correspond with an increased number of both platelet–leucocyte and platelet–platelet aggregates. Therefore, the association we observed between increased MPV and impaired microvascular perfusion could be the manifestation of platelet–leucocyte and platelet–platelet aggregate-mediated microvascular injury and endothelial dysfunction in both coronary arterioles and capillaries. These platelet-mediated effects can be expected to occur more profoundly in patients with higher platelet volumes. This suggests that MPV may be considered as a useful and independent haematological marker allowing for early and easy identification of patients who are at a higher risk of impaired reperfusion after pPCI.

Study limitations

The sample size in this study was relatively small. However, we interrogated microvascular integrity and function by using four different quantitative and objective indices to test our hypothesis. Secondly, as MPV increases in a time-dependent manner when dipotassium EDTA is used as an anticoagulant,[39] we used standardised sample tubes and collected and analysed all samples in 1 h.

Clinical implications and conclusions

The results of this study suggest that patients with high absolute and relative neutrophil counts and high MPV on admission represent the group of patients at high risk for developing microvascular reperfusion injury after recanalisation of infarct-related artery. It is possible that treatment for microvascular protection, such as glycoprotein IIb–IIIa inhibitors and thienopyridines, can be intensified and additionally using the distal protection device during the intervention can be considered in this group of patients. Also, after the intervention, the course of glycoprotein IIb–IIIa inhibitors can be prolonged.

CONCLUSION

Absolute and relative neutrophilia and higher MPV on admission were independently associated with impaired microvascular perfusion in patients with AMI treated with pPCI. It is possible that neutrophilia and high MPV are simple surrogate markers of worse microvascular injury.

ACKNOWLEDGEMENTS

Part of this study was presented at the 78th AHA Scientific Congress 2005, Dallas, USA, and was published in abstract form in Circulation 2005;112(Suppl II):3450.

Authors' affiliations

Murat Sezer, Irem Okcular, Taner Goren, Huseyin Oflaz, Yılmaz Nisanci, Berrin Umman, Fehmi Mercanoglu, Ahmet K Bilge, Mehmet Meric, Sabahattin Umman, Department of Cardiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

Competing interests: None declared.

REFERENCES

1 **Barron HV**, Cannon CP, Murphy SA, *et al*. Association between white blood cell count, epicardial blood flow, myocardial perfusion, and clinical outcomes in the setting of acute myocardial infarction. *Circulation* 2000;**102**:2329–34.
2 **Kloner RA**, Giacomelli F, Alker KJ, *et al*. Influx of neutrophils into the walls of large epicardial coronary arteries in response to ischemia/reperfusion. *Circulation* 1991;**84**:1758–72.
3 **Kawaguchi H**, Mori T, Kawano T, *et al*. Band neutrophil count and the presence and severity of coronary atherosclerosis. *Am Heart J* 1996;**132**:9–12.
4 **Martin JF**, Shaw T, Heggie J, *et al*. Measurement of the density of human platelets and its relationship to volume. *Br J Haematol* 1983;**54**:337–52.
5 **Giles H**, Smith REA, Martin JF. Platelet glycoprotein IIb–IIIa and size are increased in acute myocardial infarction. *Eur J Clin Invest* 1994;**24**:69–72.
6 **Corash L**, Tau H, Gralnick HR. Heterogeneity of human whole blood platelet subpopulations. I. Relationship between buoyant density, cell volume, and ultrastructure. *Blood* 1977;**49**:71–87.
7 **Martin J**, Bath PMW, Burr ML. Increased platelet size following myocardial infarction is associated with subsequent death and nonfatal reinfarction. *Lancet* 1991;**338**:1409–11.
8 **Huczek Z**, Kochman J, Filipiak KJ, *et al*. Mean platelet volume on admission predicts impaired reperfusion and long-term mortality in acute myocardial infarction treated with primary percutaneous coronary intervention. *J Am Coll Cardiol* 2005;**46**:284–90.
9 **Ott I**, Neumann FJ, Gawaz M, *et al*. Increased neutrophil-platelet adhesion in patients with unstable angina. *Circulation* 1996;**94**:1239–46.
10 **Neumann FJ**, Marx N, Gawaz M, *et al*. Induction of cytokine expression in leukocytes by binding of thrombin-stimulated platelets. *Circulation* 1997;**95**:2387–94.
11 **Lefer AM**, Campbell B, Scalia R, *et al*. Synergism between platelets and neutrophils in provoking cardiac dysfunction after ischemia and reperfusion. *Circulation* 1998;**98**:1322–8.
12 **Barbato E**, Aarnoudse W, Aengevaeren GW, *et al*. Validation of coronary flow reserve measurements by thermodilution in clinical practice. *Eur Heart J* 2004;**25**:219–23.
13 **Pijls NH**, De Bruyne B, Smith L, *et al*. Coronary thermodilution to assess flow reserve: validation in humans. *Circulation* 2002;**105**:2480–4.
14 **Ragosta M**, Camarano GP, Kaul S, *et al*. Microvascular integrity indicates myocellular viability in patients with recent myocardial infarction: new insights using myocardial contrast echocardiography. *Circulation* 1994;**89**:2562–9.
15 **Sakuma T**, Hayashi Y, Sumii K, Imazu M, *et al*. Prediction of short- and intermediate-term prognoses of patients with acute myocardial infarction using myocardial contrast echocardiography one day after recanalization. *J Am Coll Cardiol* 1998;**32**:890–7.
16 **Kirtane AJ**, Bui A, Murphy S, *et al*. Association of peripheral neutrophilia with adverse angiographic outcomes in ST-elevation myocardial infarction. *Am J Cardiol* 2004;**93**:532–6.
17 **Yamamoto K**, Ito H, Iwakura K, *et al*. Pressure-derived collateral flow index as a parameter of microvascular dysfunction in acute myocardial infarction. *J Am Coll Cardiol* 2001;**38**:1383–9.
18 **Sezer M**, Nisanci Y, Umman B, *et al*. New support for clarifying the relation between ST segment resolution and microvascular function: degree of ST segment resolution correlates with the pressure derived collateral flow index. *Heart* 2004;**90**:146–50.
19 **Bax M**, de Winter RJ, Schotborgh CE, *et al*. Short- and long-term recovery of left ventricular function predicted at the time of primary percutaneous coronary intervention in anterior myocardial infarction. *J Am Coll Cardiol* 2004;**43**:534–41.
20 **Katsuomi I**, Hiroshi I, Shigeo K, *et al*. Analysing coronary blood flow pattern can detect the no-reflow after anterior infarction better than other angiographic, electrocardiographic and enzymatic modalities. *Eur Heart J* 2004;**25**:1526–33.
21 **Tsunoda T**, Nakamura M, Wakatsuki T, *et al*. The pattern of alteration in flow velocity in the recanalized artery is related to left ventricular recovery in patients with acute infarction and successful direct balloon angioplasty. *J Am Coll Cardiol* 1998;**32**:338–44.
22 **Fearon FW**, Balsam LB, Farouque O, *et al*. Novel index for invasively assessing the coronary microcirculation. *Circulation* 2003;**107**:3129–32.
23 **Buffon A**, Biasucci LM, Liuzzo G, *et al*. Widespread coronary inflammation in unstable angina. *N Engl J Med* 2002;**347**:5–12.
24 **Mehta JL**, Nichols WW, Mehta P. Neutrophils as potential participants in acute myocardial ischemia: relevance to reperfusion. *J Am Coll Cardiol* 1988;**11**:1309–16.
25 **Engler RL**, Schmid-Schonbein GW, Pavelec RS. Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. *Am J Pathol* 1983;**111**:98–111.
26 **Sheridan FM**, Cole PG, Ramage D. Leucocyte adhesion to the coronary microvasculature during ischemia and reperfusion in an invivo canine model. *Circulation* 1996;**93**:1784–7.
27 **Frangogianis NG**, Youker KA, Rossen RD, *et al*. Cytokines and microcirculation in ischemia and reperfusion. *J Mol Cell Cardiol* 1998;**30**:2567–76.
28 **Simpson PJ**, Fantone JC, Mickelson JK, *et al*. Identification of a time window for therapy to reduce experimental canine myocardial injury: suppression of neutrophil activation during 72 h of reperfusion. *Circ Res* 1988;**63**:1070–9.
29 **Ma XL**, Weyrich AS, Lefer DJ, *et al*. Monoclonal antibody to L-selectin attenuates neutrophil accumulation and protects ischemic reperfused cat myocardium. *Circulation* 1993;**88**:649–58.
30 **Siminiak T**, Flores NA, Sheridan DJ. Neutrophil interactions with endothelium and platelets: possible role in the development of cardiovascular injury. *Eur Heart J* 1995;**16**:160–70.
31 **Kloner RA**, Ganote CE, Jennings RB. The ''no-reflow'' phenomenon after temporary coronary occlusion in the dog. *J Clin Invest* 1974;**54**:1496–508.
32 **Wong CK**, French JK, Gao W, *et al*. Relationship between initial white blood cell counts, stage of acute myocardial infarction evolution at presentation, and incidence of thrombolysis in myocardial infarction-3 flow after streptokinase. *Am Heart J* 2003;**145**:95–102.
33 **Tamura A**, Watanabe T, Nasu M. Association between neutrophil counts on admission and left ventricular function in patients successfully treated with primary coronary angioplasty for first anterior wall acute myocardial infarction. *Am J Cardiol* 2001;**88**:678–80.
34 **Karpatkin S**. Heterogeneity of human platelets. II. Functional evidence suggestive of young and old platelets. *J Clin Invest* 1969;**48**:1083–7.
35 **Martin JF**, Bath PMW, Burr ML. Influence of platelet size on outcome after myocardial infarction. *Lancet* 1991;**338**:1409–11.
36 **Martin JF**. Platelet heterogeneity in vascular disease. In: Martin JF, Trowbridge EA, eds. *Platelet heterogeneity:biology and pathology*. London: Springer-Verlag, 1990:205–26.
37 **Davies MJ**. A macro and micro view of coronary vascular insult in ischemic heart disease. *Circulation* 1990;**82**(Suppl II):II-38–46.
38 **Neumann F-J**, Zolnhö fer D, Fakhoury L, *et al*. Effect of glycoprotein IIb/IIIa receptor blockade on platelet-leukocyte interaction and surface expression of the leukocyte integrin Mac-1 in acute myocardial infarction. *J Am Coll Cardiol* 1999;**34**:1420–6.
39 **Bath PM**, Butterworth RJ. Platelet size: measurement, physiology and vascular disease. *Blood Coagul Fibrinolysis* 1996;**7**:157–61.